NOTES ON REGULATION IN STYLARIA LACUSTRIS.

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This paper is not concerned chiefly with regeneration, but more especially with some processes of regulation accompanying regeneration which were observed in the living animals. *Stylaria lacustris* L. is a well-known fresh-water oligochæte of the family Naididæ, whose members have the power of reproducing by self-division. The worm is six to eight mm. in length, and is easily recognized by its long prostomium or proboscis. The body is divided into three regions: (1) An anterior specialized region of five segments, containing the pharynx, which is marked by a yellow pigment distinct in appearance from the dark brown chloragogue layer of the rest of the alimentary tract. The first segment bears the long prostomium and eyes. The mouth is ventral and the pharynx is slightly eversible. The anterior region is further distinguished by the absence of the capilliform dorsal setæ, ventral setæ also being absent from the first segment. (2) The middle region, with an indefinite number of segments, containing the œsophagus and the crop, a dilatation between the seventh and eighth segments, and the stomach-intestine. The middle region ends in a budding zone of incompletely developed segments and embryonic tissue. (3) The specialized anal segment, somewhat longer than the rest and tapering in form, devoid of setæ, and with the gut lined with cilia.

Self-division takes place at a region posterior to the middle of the worm, from the seventeenth to the twenty-fifth segments, very commonly at the end of the twenty-first. The first indication of division is the appearance of a band of transparent tissue divided in the middle by a slight constriction. Embryonic tissue accumulates before and behind the septum between the segments forming an ectodermal thickening. These regions lengthen and soon become segmented, that in front of the septum forming the anal segment of the anterior zoöid, and the part behind developing into a pharyngeal region of five segments for the posterior zoöid. The zoöids remain united until completely

developed, a continuous band of fæcal matter being visible through the length of the worm. During rapid division another zone of fission makes its appearance, one segment in front of the one previously formed, making a chain of three zoöids.

This method of self-division is often compared with the more primitive type called fragmentation in which the parts separate before regenerating the ends. The physiological regeneration that takes place in self-division is the same in its results as regeneration from a cut surface. Asexual multiplication continues through the warmer months. In October and November the sexual organs attain maturity. Budding then ceases, and the power of regeneration is also diminished.

Methods.

Whole mounts of these worms have been made by the following method. As stated above, the results of this paper have been obtained from the study of the living forms, but when it has been desired to preserve specimens to show results of regeneration, difficulty was encountered in killing the animals in an extended condition, since they invariably become coiled up on application of the fixing fluid. A remedy for this was found by getting the animal extended in the angle between the beveled edge of a slide and a glass plate. A very little hot sublimate-acetic applied to the animal in this position will be drawn under the slide with considerable force and prevent the animal from moving. If as little liquid as possible be used, the animals may be killed in a perfectly extended condition. The animal should be placed on the glass plate in a small drop of water. If the slide is then moved up to the drop, the worm will become extended next to the glass, and held there with sufficient force to prevent its coiling when the killing fluid is applied.

I. Regeneration in the Asexual Forms.

Experiments. 1. A résumé of some general features of regeneration in *Stylaria.*

These forms including *S. lacustris* have been studied extensively, both in respect to their method of self-division and their regeneration. Section within the middle region is followed by

regeneration of the pharyngeal region of five segments from an anterior cut surface. Similarly, the anal segment is regenerated from a posterior cut surface, but within a shorter time. In regeneration a bud or knob of transparent embryonic tissue is first proliferated, which increases in length, develops a prostomium, and becomes segmented within a few days. Section within the anterior specialized region is followed by the restoration of the number of segments which were removed, from one to five.

Thus it is seen that the pharyngeal region of five segments is the unit of regeneration after section within the trunk region, and the process is identical with the normal or physiological regeneration that occurs in self-division. On the other hand, the segments are the units of regeneration when the mutilation is within the pharyngeal region.

There is a close analogy between these results and the observations made upon the earthworm, in which four or five segments are regenerated after more than five are cut off.

The prostomium has great power of regeneration. Frequent cases are met with of forms with regenerating prostomium. Child ('00) figured a specimen found in nature with a forked prostomium which was doubtless a product of regeneration. In Fig. 9 is shown a somewhat similar case of regeneration of the prostomium following the severing of the organ at the base; in this case the organ is doubled. In later stages the lateral bud was absorbed.

2. Regulation of the intestine behind a cut surface to form the œsophagus and crop.

It was stated at the outset that this paper deals chiefly with certain processes of regulation accompanying regeneration proper. As an excellent example of the general regulative changes accompanying regeneration in this worm a case of regulation may be mentioned which occurs normally in the process of self-division or physiological regeneration as well as in cases of regeneration after injury. The œsophagus and crop occur in the sixth to eighth segments, *i. e.*, not in the region which is formed from new tissue by proliferation. Hence the œsophagus and crop must be formed by a process of regulative transformation of the intestine behind the new proliferating tissue. This involves a

narrowing of the intestine to form the œsophagus and a dilatation to form the crop. The histological features are not considered here. The same process occurs in regeneration from a cut through the intestine.

3. Inhibition of the zone of fission under the influence of a regenerating region anterior to the fission plane.

A striking instance of regulation is seen in the effect produced upon a zone of fission by section anterior to it. Under certain conditions the formation of a regenerating region will inhibit the process of self-division and cause the disappearance of the zone of fission. This involves the rearrangement of tissues and a process of redifferentiation in a region at a distance from the cut surface. If the animal is severed in front of and near to a zone of fission which is in an early stage of development before segmentation has taken place, the constriction and the accumulated embryonic tissue disappear and the zone is completely redifferentiated (Fig. 1, *a–c*).

The nearer the regenerating region is to the zone of fission the greater is the tendency for the latter to disappear, but the influence may be exerted at a considerable distance. The following table gives the results in 48 cases:

Number of Segments Between the Cut Surface and the Zone of Fission.	Number of Experiments.	Disappearance of Zone of Fission in.
1–7	28	60 per cent.
8–15	20	15 per cent.

In all the cases included in the above table the zone of fission was in an early stage of development, but not all were at exactly the same stage. If only specimens were taken for experiment in which the zone was just beginning to form, no doubt a much larger percentage of cases of disappearance would be obtained both when the regenerating region was near and when it was far away.

The writer was informed by Professor Frank Smith of an observation upon a nearly related form, *Pristina* sp., to the effect that the zone of fission may disappear in animals kept in the laboratory, as a result of insufficient food or other unfavorable conditions. It is realized that a control experiment would be desirable, to show what percentage of cases of disappearance of

the fission zone might be traceable to other causes than the influence of the regenerating region. The results here given are, however, relied upon in the belief that the early disappearance of the zone of fission (in all cases in about two or three days) sufficiently indicated that it was due to the influence of the regenerating region. There is also the evidence of the cases to be mentioned later in which the zone of fission did not disappear subsequent to a cut behind the zone of fission.

An effect is produced the converse of what has just been described when an animal is severed in front of and near to a well-advanced zone of fission. In such a case its development exerts a retarding influence on the regenerating region. A small piece in front of such a zone of fission is soon separated, after undergoing little or no regeneration.

4. Effects of section behind a recently formed zone of fission.

Section behind a recently established zone of fission does not ordinarily seem to exert an inhibitive influence. In only one such case observed was the zone redifferentiated. No change is produced, unless it be a retardation of the development of the zone of fission. Two considerations seem to have weight in explaining the difference between this and the case in which the cut is anterior to the fission plane. First, the regeneration of the anal segment is a process requiring the withdrawal of less material from the old parts than the regeneration of an anterior end, and hence is calculated to interfere less with the development of the zone of physiological regeneration. Second, the establishment of a regenerating region behind the zone of fission does not materially change the conditions already existing in that region, since in this case a regenerating region posterior to the fission plane is substituted for the previously existing proliferating region in front of the anal segment.

Galloway ('99) has noted in respect to *Dero vaga* that cutting behind the fission zone tends to disorganize its growth and the posterior piece soon drops off.

As a parallel to the results of the preceding experiments upon *Stylaria* it may be mentioned that Dr. Child has informed me that he has found a similar disappearance of the fission plane in the Rhabdocœle *Stenostoma*, under the influence of a regenerating

region. There is probably a close similarity in the physiological processes connected with fission in the two groups.

5. Disorganization in the region behind a cut surface.

(*a*) There are a number of phenomena taking place internally during regeneration which can be plainly seen, owing to the transparency of these animals. When a portion of the head segment in front of the eyes is severed, in almost all cases the pigmented portions of the eyes are found later in a fragmented condition and the black pigment cells scattered through the pharyngeal region (Figs. 2, 5, 11). This does not seem to be a direct mechanical effect, since it occurs when the eyes are apparently not directly injured by the mutilation. This observation, which may seem of slight importance, is mentioned as showing how organs behind the regenerating region may be affected, apparently disorganized, in this case, and later restored. The eyes are reformed subsequently and the scattered pigment cells disappear. In Figs. 5 and 11 the eye appears in two parts. It became normal later. The interpretation, if such it may be called, which would seem to be indicated by the facts, is that the region just behind the cut surface tends to return to an embryonic or disorganized condition as the result of change in physiological conditions.

(*b*) Another observation of a like nature has been made in the case of the regeneration of the whole pharyngeal region. During the regeneration numerous brown pigmented cells, similar to the chloragogue cells of the stomach-intestine, are seen floating in the cœlomic fluid. These are different from the normal unpigmented cœlomic corpuscles. As was pointed out above, the region behind the cut surface is a seat of regulative activity, the œsophagus and crop being produced in that region by regulation of the intestine. The free pigment cells may be a product of the disorganization of the intestine immediately behind the cut surface, as in the case of the pigment cells of the eye.

As stated above, these two observations seem to indicate that the organs just behind the cut surface tend to return to an embryonic condition similar to that of the new proliferated material. This breakdown of tissues may also be comparable to the loss of cell boundaries and return to a syncytial condition in the well-

known experiments of Loeb with segmenting *Fundulus* eggs, in which the cause ascribed was lack of oxygen.

6. Healing of a cut surface without regeneration.

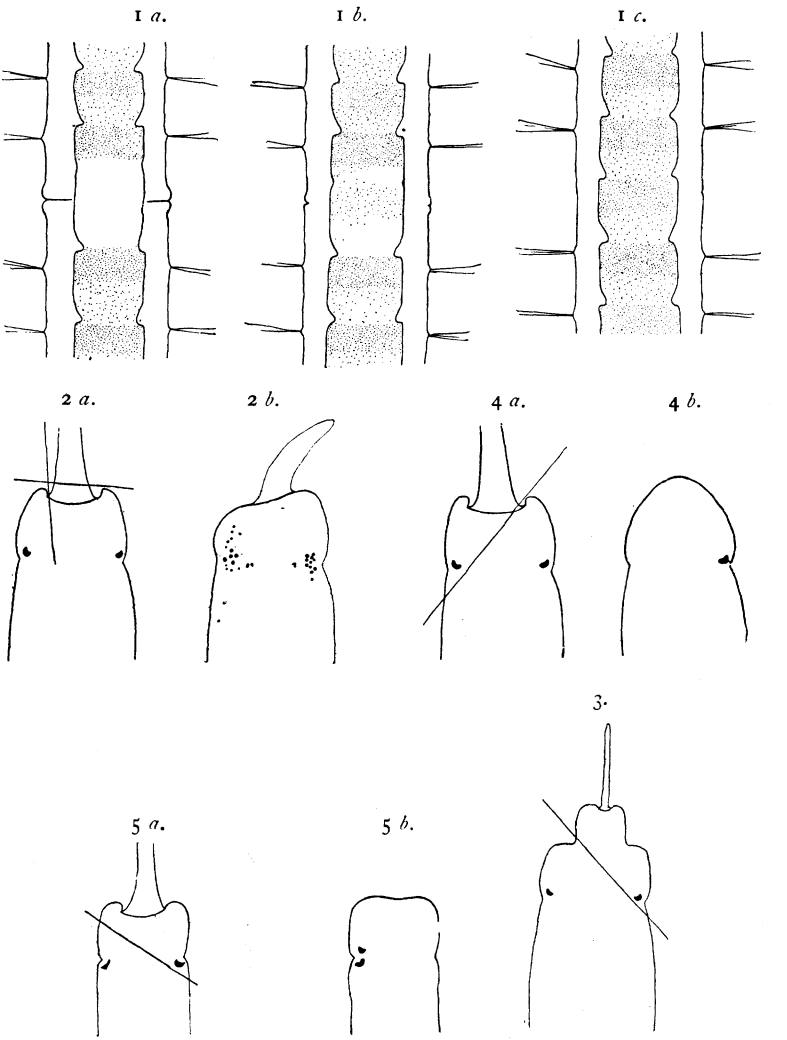
The regenerative power is so strong in the asexual naïds that it would naturally be expected that regeneration would take place under all circumstances in pieces above a certain limit of size. Certain instances of failure to regenerate have been met with, however, which indicate that the power to regenerate is dependent upon certain internal conditions. If an oblique cut be made in front of the pharynx, removing the prostomium and one side of the head segment, including perhaps one eye, the cut surface may frequently heal over and failure to regenerate the prostomium may result (Figs. 4 and 5). A number of such cases have been obtained. In some instances the animals were kept for more than three weeks without showing any tendency to regenerate the prostomium. Since regeneration ordinarily requires only a few days, the effects seemed in these cases to be permanent.

Internal conditions favorable to proliferation, such as the exposure of cut surfaces of intestine and blood-vessels, are present in nearly all possible experiments with *Stylaria*. But after section of the prostomium and one side of the head without touching the pharynx, the tendency to proliferate is apparently at a minimum. The ectoderm may, therefore, close over the wound and cut off the outlet for proliferating material. Failure to regenerate seemed to follow only after an oblique cut, but in one instance, after a transverse cut in front of the eyes, a somewhat similar result was obtained. In this animal an outgrowth appeared from the cut surface which was of less diameter than the region behind, appearing like a new head segment superimposed upon the old. Its appearance suggested that the outlet for the proliferating material had been narrowed by reduction of the cut surface. It is conceivable that still further reduction of the cut surface, or even complete closing of the wound, might occur in case the proliferating tendency was slight (Fig. 3).

The above cases of failure to regenerate were only occasional instances, showing that the balance between the tendencies to proliferate and to heal over without regeneration is rather deli-

cately adjusted. Cases occurred in which the prostomium was regenerated, but the side of the head or lobe was not normally restored, at least within the period of observation (cf. Fig. 13, of a sexual individual).

The general conclusion is that certain parts which may be mutilated without exposing cut surfaces of internal organs which are of importance as internal factors in proliferation are less likely to be regenerated, since, when the proliferating tendency is slight, the ectoderm closes over the wound and checks proliferation.



DESCRIPTION OF FIGURES.

FIG. 1. (*a*, *b*, *c*.) Redifferentiation of a zone of fission. In (*a*), a zone is formed and there is a constriction in the body-wall. The intestine in this region is transparent, devoid of pigment. In (*b*) and (*c*) the zone and constriction have disappeared and the intestine becomes pigmented. The accumulation of transparent embryonic tissue before and behind the septum in (*a*), mentioned in the text, is not indicated in the figure. The ectodermal thickening is indicated at the constriction. In this and all the figures the dorsal capilliform setæ are not represented full length.

FIG. 2. Fragmentation of the eyes after mutilation. The prostomium was severed and a short longitudinal cut made in the first segment. The direction of the cut indicated by line. The condition in (*b*) was observed three days after the mutilation. The slightly curved outgrowth of the bud of the prostomium is an unusual occurrence.

FIG. 3. Regeneration from first segment, resembling a repetition of the head segment, of less diameter. The line indicates direction of the cut. The eye is regenerated. The condition here represented was fifteen days after the operation.

FIG. 4. Failure of regeneration after an oblique cut through first segment. In (*b*) the condition twenty-three days after the operation is shown. The eye is not regenerated and there is no trace of a prostomium.

FIG. 5. Failure of regeneration in first segment. The eye in (*b*) is fragmented into two portions. Later it became normal.

7. Absence of regeneration in short posterior pieces.

With the purpose of finding whether heteromorphosis would take place, as in the earthworm, in the case of very short posterior pieces a number of experiments were tried with as short posterior pieces as would survive the operation. No tails appeared on the anterior ends. A number of pieces which survived the operation for as long a time as three weeks, did not regenerate the anterior ends. During the first week these pieces elongated within the budding region in front of the anal segment. The immature segments became further differentiated, their setæ increasing in size, but no new segments appeared. The pieces then appeared to be composed of segments nearly equal in size, and there was no zone of embryonic undifferentiated tissue remaining. One such piece was obtained by a cut one segment behind the pharynx of a posterior zoöid. It contained at first a region composed chiefly of indistinct segments. At the end of a week these segments had increased in length and their setæ had grown until twenty segments could be easily counted. Its size was greater than that of a minimal piece capable of regeneration taken from the middle of the body. It showed no trace of anterior regeneration for three weeks, but was still active in its movements. Un-

fortunately, it was not possible to keep it under observation longer. A number of similar pieces have been kept with the same results (Figs. 6 and 7). The elongation of such pieces did not seem to be merely mechanical, since it was accompanied by some differentiation such as the growth of the segments. Possibly the proliferating tendency at the cut surface was checked by the continued growth of the budding zone. The latter, containing an accumulation of embryonic tissue, was perhaps able to check regeneration by absorbing the available materials for growth. Whether such pieces would in time regenerate needs, of course, to be determined before the possibility of heteromorphosis would be settled.

8. Regeneration of the posterior end.

It was thought possible that heteromorphosis might occur in regeneration from short anterior pieces. In no case did the specialized anterior region of five segments survive long enough to begin regeneration. An anterior piece of eight segments survived for six days without showing any posterior regeneration. This was the shortest piece which was able to survive more than a short time after the operation. Pieces with only two more of the trunk segments present on the average regenerated freely, however, as the following table will show:

Length.	Number of Experiments.	Number Able to Survive for Several Days.	Number Able to Regenerate.
8–9 segments.	9	2	0
10–11 "	10	9	7
12–13 "	10	9	9
14–29 "	37	35	33

There is a great average increase in the power to regenerate correlated with the presence of only two additional segments of the trunk. The specialized head region, as would be expected, has the least power of regeneration. Portions of the trunk of the same length as the first class given in the above table are able to regenerate freely.

No cases of true heteromorphosis were obtained. The anterior region of five segments which might perhaps be expected to develop a head posteriorly, contains no nephridia (they are found back of the sixth segment), and as we have seen, does not survive the operation long enough to regenerate. Results obtained in

Planaria, with its diffuse excretory system, could not be expected to occur in a form like *Stylaria*, with a head region so specialized as to be unable to exist independently even for a short time.

A case of duplication of parts was seen in the production of a double-headed individual, one of whose heads was larger and in line with the axis of the trunk. The other was lateral, lying close to it, and smaller. The smaller one was in process of absorption at the last stage at which it was observed (Fig. 8).

A doubling of the prostomium was noticed in one case as mentioned above. The two organs lay parallel and were fused together except at the tip of the shorter (Fig. 9). In later stages the lateral bud was absorbed.

9. Regulation of growths oblique to the main axis.

It is a common observation that proliferation tends to take place along an axis normal to the cut surface. After an oblique cut the new growth continues oblique until it has reached its full length or nearly so. The straightening of such oblique growths is evidently not a function of growth alone, but of growth influenced by certain tensions, which may be exerted by the part behind the new growth. Rievel ('96) has pointed out the influence exerted by the peristaltic motions of the intestine upon the regeneration of the head region. The knob of new tissue is at first solid and is later penetrated by the slower ingrowth of the lumen of the intestine into the region. The waves of peristaltic motion constantly passing along the intestine favor its ingrowth into the new region. Finally the mouth is formed by the bulging out of the body-wall, caused by peristalsis. It is broken through from within, there being no stomodæal invagination of the ectoderm in regeneration.

It may be suggested that peristaltic movements of the intestine have an influence in straightening oblique growths. This is probable since the straightening occurs not during the proliferating period, but coincidently with the ingrowth of the pharynx. Straightening occurs, moreover, while the tissue still appears embryonic before the complete differentiation of muscular and nervous systems (Figs. 10 and 13).

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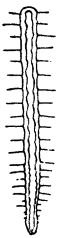

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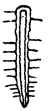

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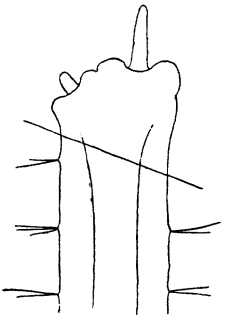

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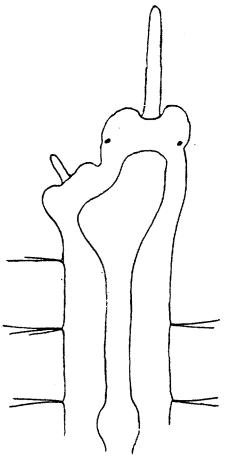

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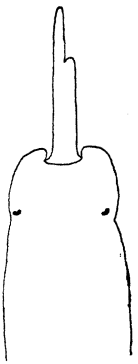

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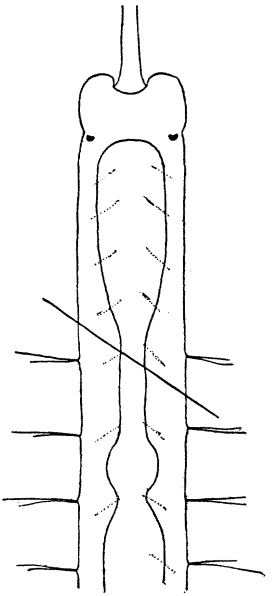

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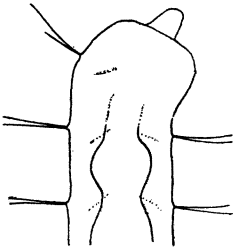
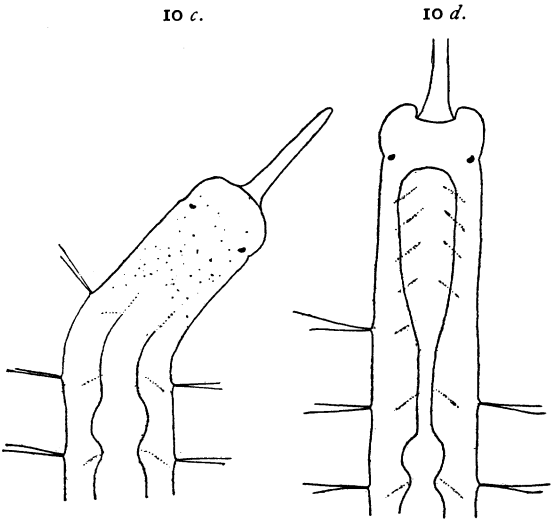




DESCRIPTION OF FIGURES.

FIGS. 6 and 7. Failure of anterior regeneration in case of very short posterior pieces. Fig. 6 represents such a piece twenty-three days after it was severed, Fig. 7 a piece eighteen days after it was severed. It measured 1.25 mm. Further observation was prevented.

FIG. 8. Regeneration of a double-headed individual. The line in (*a*) indicates direction of cut. The smaller head was apparently destined to be absorbed at last observation. The specimen was lost before regeneration was complete. Stages (*a*) and (*b*) were six and ten days after the cut was made.

FIG. 9. Regeneration of abnormal prostomium, a doubling of the organ. Later the lateral bud was absorbed.

FIG. 10. Regeneration occurring in a direction normal to the cut surface. (*b*) represents the condition three days after the operation, (*d*) the complete regeneration. In (*d*) the right dorsal bundle of setæ is not restored. In (*c*) scattered pigment cells resembling the chloragogue cells are present in the new tissue.

II. REGENERATION OF THE SEXUAL FORMS.

The sexual organs of *Stylaria* come to maturity during the months of October and November. In the height of the sexual stage the animals cease to multiply asexually. In the earlier stages budding animals are frequently seen. In the fully developed sexual animal the formation of new segments in the anal proliferating region also ceases. As a result the animals attain larger size, since the segments in the budding region become fully differentiated. The clitellum is conspicuous, covering the fifth and sixth segments, in which the testes and ovaries lie. This part becomes more opaque and loses its setæ.

The results obtained in regard to the regeneration of the sexual forms will be treated under two heads: (1) The regeneration of the pharyngeal region involving the results following the severing of 1–5 anterior segments; (2) regeneration within the trunk region.

1. In the regeneration of the pharyngeal region three sorts of cases are met with:

(*a*) Complete regeneration may occur in the less mature sexual forms.

(*b*) There may be an incomplete regeneration, resulting in the production of an outgrowth which is devoid of ventral setæ and seems to be the equivalent of the first segment. The regenerated part is distinguishable by its relative lack of pigment and by the fact that it does not attain the original diameter (Fig. 11). It is comparable to Fig. 3, where a growth took place in an asexual form from an apparently restricted outlet, and the same explanation may be offered here, viz., that owing to the slowness of proliferation the partial closure of the cut surface by the body-wall restricted the outlet. The prostomium does not reach full length and the lateral lobes of the head are not developed. Often the eyes are not formed in such cases.

(*c*) Often complete failure to regenerate occurs. The end heals over and a mouth is formed (Figs. 12 and 14). In twenty-one cases of mature sexual animals in which from one to four segments were removed there were six such cases of failure to regenerate and the rest regenerated an amount apparently equivalent to one segment.

2. After the removal of five or more segments results followed which likewise may be divided into three classes.

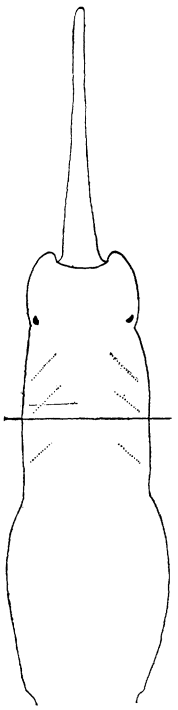
(*a*) Normal regeneration in the less mature sexual specimens.

(*b*) A pharyngeal region of five very short segments with a diminutive prostomium may be formed. Four pairs of ventral setæ appear showing that the full number of segments is represented, but the region is remarkable for its dwarfed appearance (Fig. 15).

(*c*) There may also be failure to regenerate. In twenty-nine cases in which from six to thirty segments were removed there were six cases of failure to regenerate anteriorly and five cases of the dwarfed regeneration.

The regeneration of the anal segment is equally slow in the advanced sexual forms and the part regenerated is not of full size (Fig. 16).

3. In a single instance the clitellum was regenerated, but the specimen was unfortunately not kept under observation long



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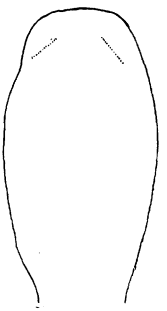

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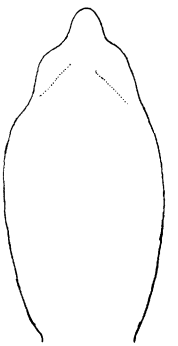

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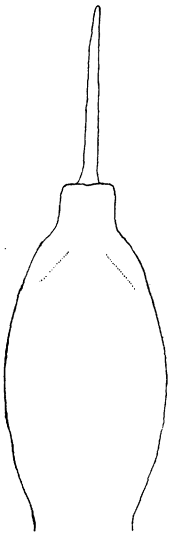

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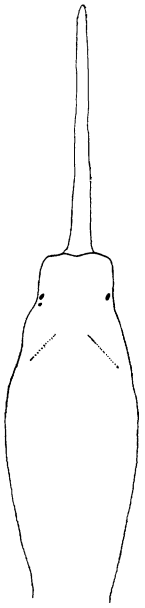

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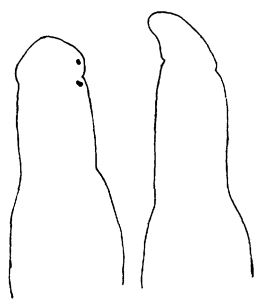

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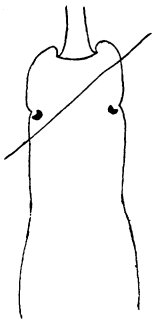

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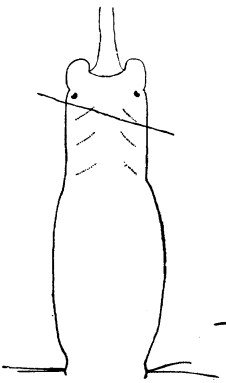

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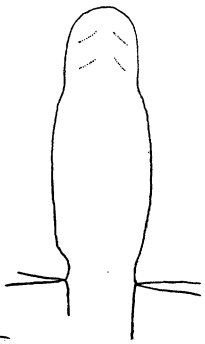
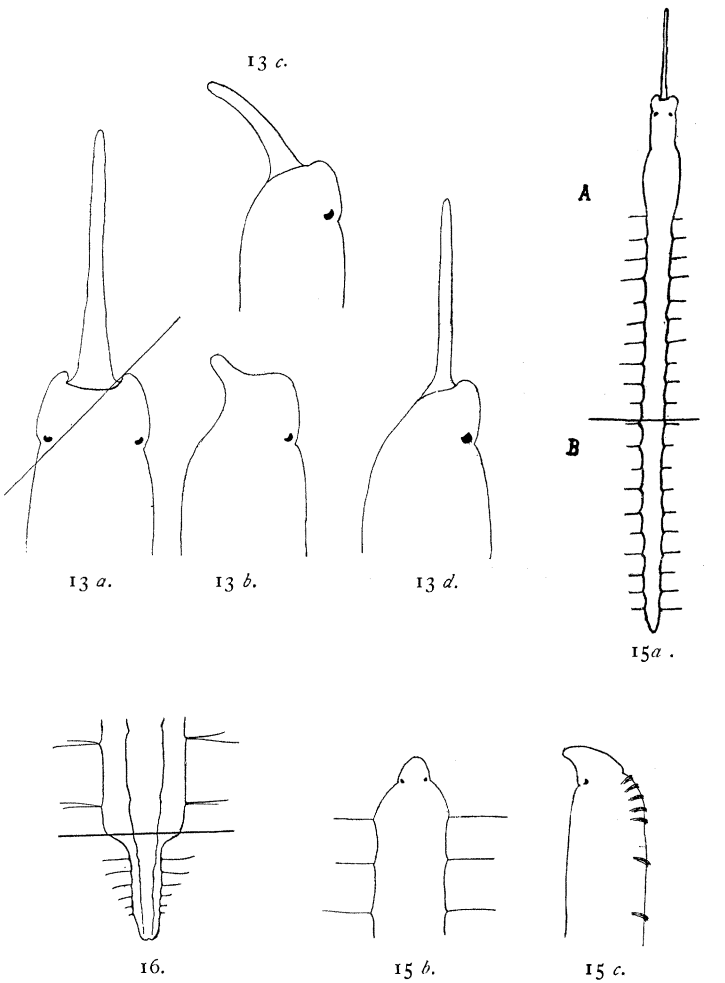


14 *b*.



DESCRIPTION OF FIGURES.

FIG. 11. Regeneration of a sexual individual after removal of first three segments. The clitellum is indicated by the widening of the body. It takes in the fifth segment, which is a part of the pharyngeal region. The setæ are absent from the clitellum. One pair of ventral bundles were left in front of the clitellum. In stage (*e*) the head segment is regenerated. As no more pairs of setæ bundles were produced, the regenerated part is regarded as the equivalent of the head segment alone. The stages (*b–e*) followed 3, 8, 12 and 19 days after the operation, respectively.

FIG. 12. Failure of regeneration after severing part of first segment in a sexual individual. In (*b*) a side view is given. The condition shown in (*b*) and (*c*) followed seventeen days after the cut.

FIG. 13. Oblique growth of bud of prostomium in (*b*), and slightly incomplete regeneration. Stages (*b*, *c*, *d*) were 4, 8 and 18 days after the operation, respectively. The eye was not regenerated. The other eye increased in size.

FIG. 14. Failure of regeneration in a sexual individual after removal of two segments. This condition existed nineteen days after cut was made.

FIG. 15. Dwarfed anterior regeneration from a sexual individual after removal of fifteen segments. The prostomium is not regenerated. A pharyngeal region of five short segments is produced, as shown by the number of setæ bundles. In (*c*) a side view is given. The level at which the cut was made is shown in (*a*).

FIG. 16. Regeneration from posterior end of a sexual individual. The part produced is of less diameter.

enough to determine whether the regeneration of the sexual organs was complete. The cut was made just behind the clitellum.

CONCLUSIONS.

1. The formation of a regenerating region will under certain conditions inhibit the process of asexual multiplication and cause the disappearance of the zone of fission. This effect may be produced by a cut anterior to the zone of fission, less often by a cut posterior to it, and occurs only when the zone is embryonic. The zone is also more likely to disappear if the cut is near to it. The band of transparent embryonic tissue redifferentiates and the energy of growth is transferred to the regenerating region.

2. There is evidence of disorganization and a return to embryonic conditions in organs just behind a cut surface. This effect does not appear to be a direct mechanical result, *i. e.*, due to crushing. Fragmentation of the pigmented portion of the eye is one case adduced.

3. Internal conditions favorable to proliferation, such as the exposure of cut surfaces of intestine and blood-vessels, are present in nearly all possible experiments. But if a corner of the head segment be removed, including the prostomium, without injuring the pharynx, the ectoderm may close over the surface and regeneration may fail to take place.

4. Short posterior pieces often fail to regenerate anteriorly, but no cases of heteromorphosis, such as individuals with tails on the anterior end, have been obtained. Short posterior pieces which failed to regenerate within the time of observation may elongate and show some differentiation in the budding region.

5. The middle portion of the body has the greatest power of regeneration, the specialized pharyngeal region has the least.

6. Growth takes place at right angles to a cut surface, and if the cut is oblique the bud will grow out at an angle to the axis of the body. Straightening is affected after the penetration of the lumen of the pharynx into the region, probably under the influence of the tension produced by the peristaltic motions of the intestine.

7. Sexual individuals lose the power of regeneration to a large extent just as they do their power of budding. In an advanced sexual stage regeneration may fail to occur or be incomplete.

I wish to express my thanks to Dr. C. M. Child for suggesting to me the subject of this paper and for further suggestions relative to some of the results described.

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